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Antibody response to plague
vaccination in humans as assayed
by staphylococcal radioimmune
precipitation (St-RIP) test*

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The staphylococcal radioimmune precipitation (St-RIP) test was applied to sera of laboratory personnel who had been vaccinated against plague. Antibodies were detected in the majority of 117 sera from 55 human vaccine recipients; a few individuals appeared to be immunologically nonresponsive since they failed to produce detectable antibodies. Highly significant statistical correlations were observed among St-RIP, indirect hemagglutination, and mouse protection index tests. Increases in antibody level were usually observed upon primary and booster vaccination.

INTRODUCTION

Plague remains a disease of concern in several parts of the world; since the turn of the century it has spread among wild rodents in the Western United States, posing an increasing hazard to the human population. Anti-plague vaccines have been developed, but in the U.S., vaccination has been limited to overseas-bound military personnel and other high-risk populations. Serologic responses to vaccines and their major antigen, Fraction I, and the efficacy of plague vaccines, have been reviewed (Cavanaugh *et al.*, 1974; Marshall, Bartelloni, Cavanaugh, Kadull & Meyer, 1974; Marshall, Cavanaugh, Bartelloni & Meyer, 1974; Meyer, Cavanaugh, Bartelloni & Marshall, 1974; Meyer, Hightower & McCrumb, 1974; Meyer, Smith, Foster, Marshall & Cavanaugh, 1974).

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Older laboratory methods to monitor plague antibody levels include indirect hemagglutination (HA) and complement fixation (CF) as *in vitro* serologic tests; there is also an *in vivo* test, the mouse protective antibody index (MPI), based on the survival times of mice following injection of serum from a vaccinee and challenge with a lethal dose of plague organisms (Marshall, Cavanaugh *et al.*, 1974). Newer methods include hemagglutination inhibition (Williams, Atas & Cavanaugh, 1976) and enzyme-linked immunosorbent assay (Cavanaugh *et al.*, 1979). We report here a promising new radioimmune assay system using staphylococcal protein A as an immuno-adsorbent and *Yersinia pestis* Fraction IB as the radiolabeled antigen. Fraction IB (the carbohydrate-free crystallizable protein from Fraction I [Baker *et al.*, 1952]) is easily radiolabeled with ^{125}I , and its use in staphylococcal radioimmune precipitin (St-RIP) tests has been demonstrated for monitoring antibodies in mice used for testing the efficacy of vaccines (unpublished). In this paper we report the application of St-RIP to the detection of antibodies in human sera from laboratory personnel who had received one or more, sometimes many, doses of plague vaccine, and the correlation of St-RIP results with those of HA and MPI.

St-RIP assay exploits the immunoglobulin-binding capability of protein A located on *Staphylococcus aureus*. Immunoglobulins, particularly IgG's, of most mammalian species exhibit this phenomenon. However, some classes or subclasses, human IgG³ for example, do not react with protein A (Brunda *et al.*, 1977). The assay involves the reaction of radio-labeled antigen with specific antibody in test serum followed by the reaction of antibody-antigen complexes with protein-A-bearing staphylococci. After centrifugation, the presence of specific antibody is demonstrated by the presence of radioactive antigen in the precipitate (complexes of staph-ab-ag); unreacted antigen remains in the supernate. The percentage of antigen precipitated (% St-RIP) indicates the relative level of antibody in the test serum. The technique and variations of it have been utilized for the detection and quantitation of diverse antibodies (Brunda *et al.*, 1977; Brunner *et al.*, 1977; Habermann, Horvath & Schaeg, 1977; Jahrling, Hesse & Metzger, 1978; Soergel, Schaffer, Sawyer & Prato, 1978; Ulstrup, Figenschau & Vellar, 1974) and antigens (Kaden, 1977; Kessler, 1975; Jonsson & Kronvall, 1974) and application to additional antigen-antibody systems may be anticipated.

MATERIALS AND METHODS

Sera

A total of 117 sera from 55 individuals who had received killed (formaldehyde treated whole cells) plague vaccine USP (Cutter Laboratories, Berkeley, Ca.) (Marshall, Bartelloni *et al.*, 1974) were tested. Some of the individuals had not previously been vaccinated; others had received multiple vaccinations. All had been vaccinated as a protective measure in connection with their work in or proximal to laboratories where work with virulent plague organisms was carried out. Sera had been obtained routinely from these individuals to monitor their immune status. The sera were from three serum banks: (1) 53 sera collected during a recent two-year period from 19 Naval Biosciences Laboratory (N.B.L.) personnel; (2) 36 sera from 11 Fort Detrick personnel included in the study of Marshall, Cavanaugh *et al.* (1974) and (3) 28 sera from 25 Cutter Laboratories personnel, 12 from long-term storage (up to 11 years), and 16 from current workers.

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Antigen

The source of antigen was purified plague Fraction IB (Baker *et al.*, 1952), prepared at N.B.L. in 1971 as lyophilized antigen and submitted as reference material to the Division of Biologics Standards, N.I.H., Bethesda, Md. Reconstituted antigen (100 μg per 100 μl water) was clarified and labeled with ^{125}I by the chloramine-T method (Greenwood, Hunter & Glover, 1963; Soergel *et al.*, 1978), dialyzed and clarified. A typical antigen preparation yielded ca. 180 000 ct/min μl^{-1} . This was diluted for storage so that a convenient volume (ca. 0.1 μl per sample) yielded adequate radioactivity throughout three half lives (i.e. a minimum of ca. 1000 ct/min 0.1 μl^{-1} at ca. 6 months). This permitted the use of a standard amount of antigen per sample (ca. 0.024 μg) in St-RIP assays for this period of six months. (St-RIP is most sensitive when performed with the least amount of antigen of highest specific activity.) Radioiodinated Fraction IB reacted in the St-RIP assay with anti-Fraction IB and anti-Cutter vaccine hyperimmune rabbit sera (both kindly provided by Dr Daniel Eisler), yielding sigmoid-shaped titration curves when % St-RIP was plotted as a function of the log of serum dilution. These curves, which were similar to those previously obtained using viral antigens and homotypic antisera (Soergel *et al.*, 1978), revealed detectable reactions with the hyperimmune sera at dilutions of 1:10 000. The 50% St-RIP reactions occurred at dilutions of 1:1200 and 1:550 with anti-IB and anti-vaccine sera, respectively. Polyacrylamide sodium dodecyl sulfate gel electrophoresis (Laemmli, 1970) of ^{125}I -Fraction IB revealed a single homogeneous polypeptide of 15 000 mol. wt (Fig. 1).

St-RIP test

The procedure for St-RIP test was essentially as described (Soergel *et al.*, 1978). Briefly, 10 μl of test serum at a dilution of 1:10 or greater (or 1 μl of undiluted serum) was added to 200 μl phosphate buffered saline containing 1 μl normal rabbit serum in a

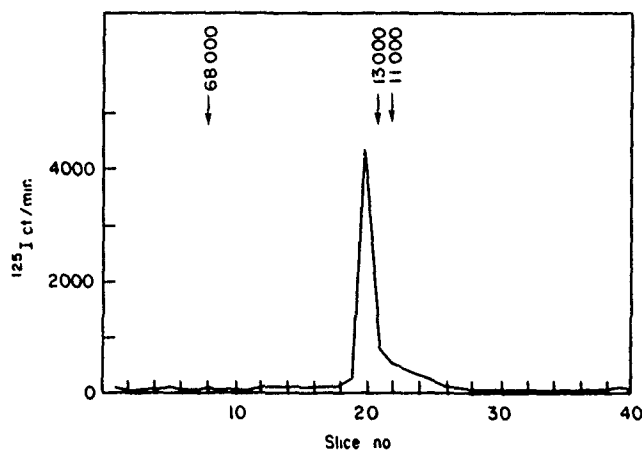


Fig. 1. Polyacrylamide gel electrophoresis of ^{125}I -labeled *Yersinia pestis* Fraction IB. A 2 μl sample of a labeled antigen preparation was subjected to SDS-disc gel electrophoresis in a 15% acrylamide gel, essentially as described (Laemmli, 1970). Protein markers, bovine albumin (mol. wt 68 000) and alpha-chymotrypsin I (subunit mol. wt 13 000 and 11 000), indicated by arrows, were stained and gels were sliced with a Hoefer gel slicer (Hoefer Scientific Instruments, San Francisco, Ca.). Individual slices were analyzed for radioactivity.

1.5 ml microcentrifuge tube; 50 μ l of radioactive standardized antigen (approximately 1000–10 000 ct/min) was added and the mixture was incubated at 37 °C for approximately 1.5 h, followed by the addition of 0.1 ml of a 5% suspension of protein A-bearing staphylococci (Pansorbin,[®] Calbiochem-Behring Corp., La Jolla, Ca.) in 0.5% Tween 20; after mixing, and 5 min incubation at room temperature, samples were centrifuged to separate supernates from precipitates, which were counted in a gamma counter.

Usually, serial tenfold dilutions of serum were assayed, and the % St-RIP, i.e. the percentage of radioactive antigen precipitated, was calculated for each serum dilution. These individual values were recorded in the Tables in order to specifically illustrate each assay result; however, when a single composite value was more appropriate (e.g. Figs 2–5), the dilution corresponding to 50% St-RIP, i.e. the reciprocal of the serum dilution yielding precipitation of 50% of the radioactive antigen, was used. Since the midrange (steepest) portion of the sigmoid-shaped curves approached linearity, the dilution for 50% St-RIP was estimated by interpolation between the nearest values greater and less than 50%. (The midrange slopes for 11 titrations of the anti-IB hyperimmune rabbit serum and 71 titrations of human sera were -33 ± 5 and -32 ± 7 % St-RIP/log dilution, respectively.) When the value was less than 50% at 1:10, this value was used in estimation of 50% St-RIP by extrapolation along a sigmoid-shaped curve derived from the anti-IB serum curve, appropriately adjusting the log dilution axis. Sera with < 19% St-RIP at the 1:10 dilution (50% St-RIP < 2) were considered antibody-negative.

Hemagglutination (HA) tests

HA tests employed aldehyde-stabilized sheep red blood cells sensitized with Fraction I (Rust *et al.*, 1972) and were conducted in microtiter plates by recommended procedures (W.H.O. Expert Committee on Plague, 1970) with twofold serum dilutions from 1:8 to 1:8192. Titers recorded were initial serum dilutions showing 4+ hemagglutination.

Mouse protection antibody index (MPI)

This test was performed according to the method of Meyer & Foster (1948). As a routine test, MPI was applied only to sera from N.B.L. personnel.

RESULTS

Comparison of St-RIP and HA

Data for all vaccinated individuals are shown in Fig. 2; not included are data on ten pre-immunization sera which were negative by both tests. As shown in the lower left hand block, 20 specimens were negative by both tests. Four negative specimens from Ft. Detrick were from one individual, and 12 from N.B.L. were repeated bleedings from three individuals. (The MPI test also showed these three individuals to be negative antibody responders.) Of the 68 sera showing positive antibodies by both tests, there was a highly significant correlation ($P < 0.01$) between St-RIP and HA. The correlation coefficient for these sera (not including the five sera with off-scale titers, HA > 8192) was 0.673. Only two sera marginally positive (1:8) by HA were negative

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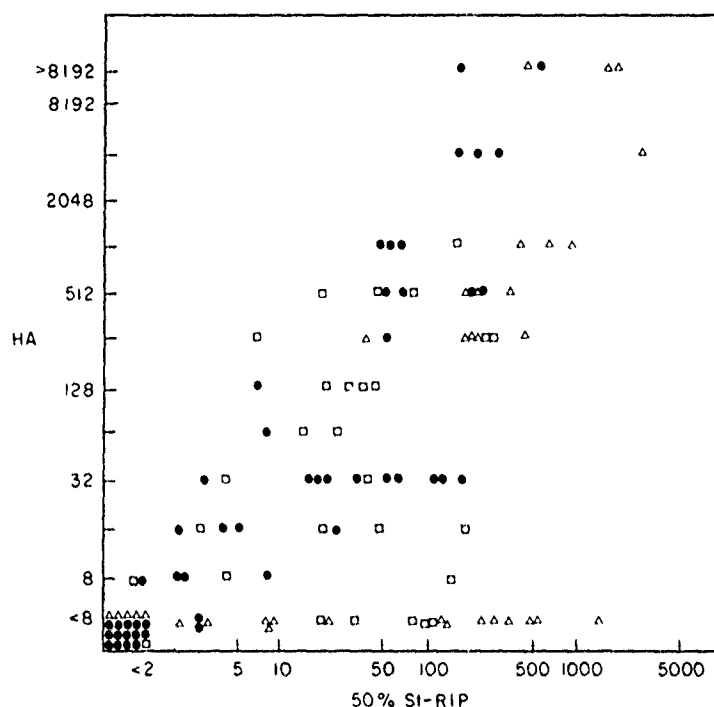


Fig. 2. Relationship between staphylococcal radioimmune precipitation (St-RIP) and indirect hemagglutination (HA) titers of sera from vaccinated individuals. Each point represents an individual serum: ●, personnel from Naval Biosciences Laboratory; Δ, personnel from Fr. Detrick; and □, personnel from Cutter Laboratories (See Materials and Methods for definition of 50% St-RIP.)

by St-RIP test. On the other hand, 21 sera considered positive by St-RIP test were negative ($<1:8$) by HA.

Figure 3 shows the chronological relationship of HA and St-RIP on sera from the individual for whom we have the most data, spanning over ten years. He maintained a high titer evidenced by both tests throughout this period, and the fluctuations seen were in parallel. It is likely there were other fluctuations related to vaccinations where serum samples were not available to us, i.e. 1955-57, 1958-62. As in the report by Marshall, Cavanaugh *et al.* (1974), serial specimens from some individuals, repeatedly vaccinated, never showed a positive reaction; others showed rises and falls in titers within a high or intermediate range.

Comparison of St-RIP and MPI

MPI determinations were made on sera from only one of the laboratories (N.B.L.). Since the numerical value of MPI decreases with an increasing level of proteic antibodies, a negative slope was observed upon relating St-RIP to MPI (Fig. 4). Although there was considerable scatter of the points, a highly significant ($P < 0.001$) correlation coefficient, $r = -0.788$, was observed for those sera showing positive St-RIP reactions. All of the St-RIP negative sera (which included eight pre-vaccination samples) had MPI values >10 , which by accepted criterion (Meyer, Smith *et al.*, 1974) were also negative by MPI. Of those sera positive by MPI (≤ 10), all but one showed

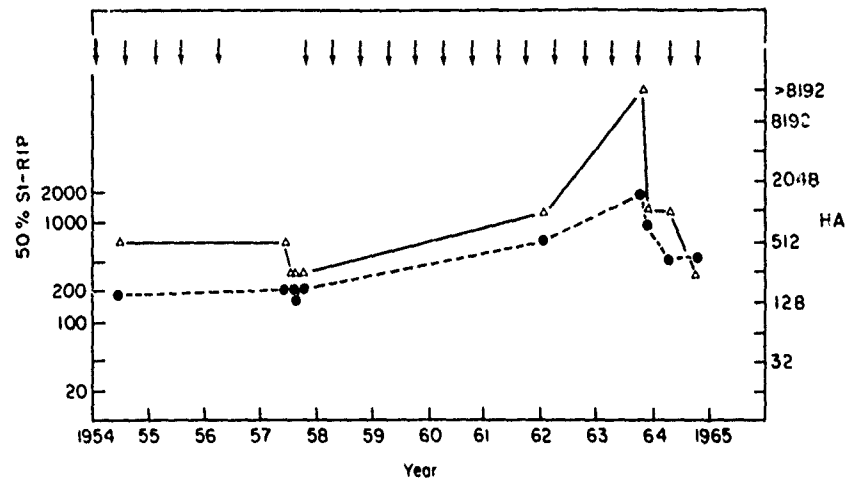


Fig. 3. Relationship between staphylococcal radioimmune precipitation (St-RIP), ●, and indirect hemagglutination (HA), Δ, in multiple serum samples from one individual from Ft. Detrick. Heavy arrow indicates time of primary vaccination (3 doses of 0.5 ml to 1 ml at 2-week intervals). Light arrows indicate booster doses of 0.25 ml vaccine (See Materials and Methods for definition of 50% St-RIP.)

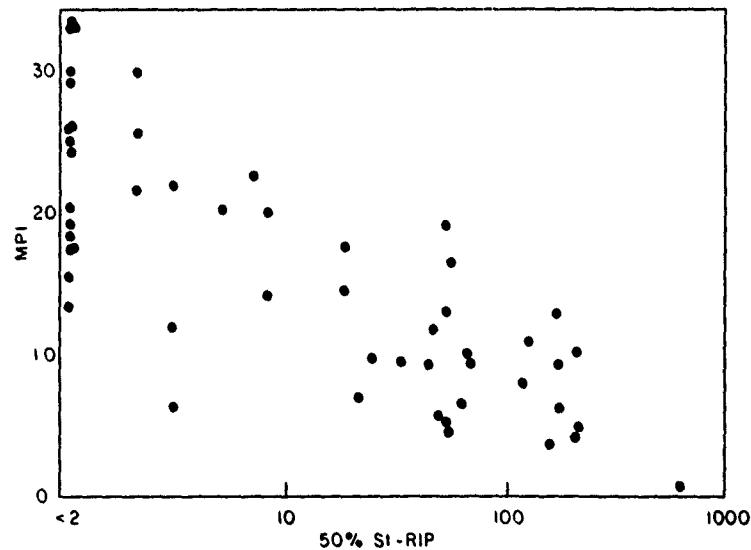


Fig. 4. Relationship between staphylococcal radioimmune precipitation (St-RIP) and mouse protective index (MPI) titers of individual sera. Each point represents an individual serum from Naval Biosciences Laboratory personnel. (See Materials and Methods for definition of 50% St-RIP.)

high St-RIP values (50% St-RIP greater than 1:20). On the other hand, many sera with appreciable St-RIP titers would be considered negative by MPI.

As with hemagglutination, MPI tests on serial bleedings indicated that some individuals were nonreactors, while other individuals reacting positively to vaccination showed rises and falls. The chronological pattern of St-RIP and MPI is shown in Fig. 5 for the individual for whom the most data are available. There was a marked positive

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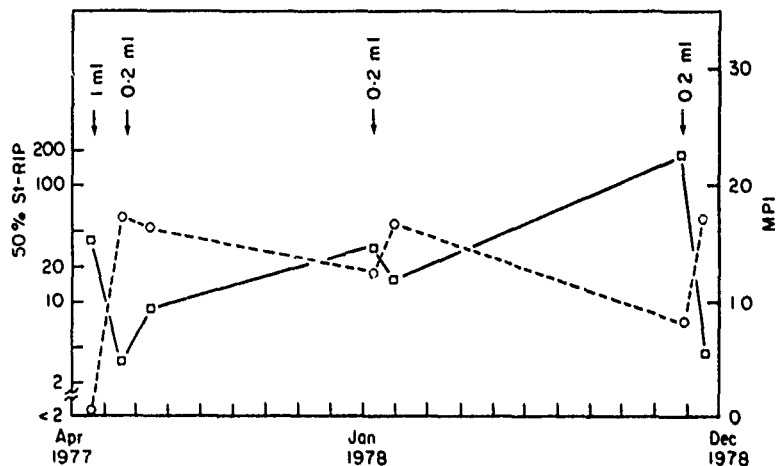


Fig. 5. Relationship between staphylococcal radioimmune precipitation (St-RIP), O, and mouse protection index (MPI), □, in multiple serum samples from one individual (No. 36) from Naval Biosciences Laboratory. Quantities and intervals of vaccinations are indicated with arrows. (See Materials and Methods for definition of 50% St-RIP.)

response following the initial vaccination and further responses upon booster immunizations. Here, again, the reciprocal relationship between St-RIP and MPI is evident, but several of the sera would be considered negative (>10) by MPI.

Response to primary vaccination

Table 1 shows results from eight individuals (all N.B.L.) for whom data were obtained by all three tests on sera drawn before and after initial vaccination. All prevaccination sera were negative by all three tests. Only two persons (Nos. 31 and 35) showed a definitive MPI response to primary vaccination; they also showed increases in St-RIP and HA titers. Two individuals, Nos. 30 and 37, failed to respond to initial vaccination (they also failed to respond to booster vaccination, Table 2). The other four persons showed varying increases in St-RIP and HA titers; changes in their MPI titers were in the appropriate direction for an antibody increase.

Response to booster vaccination

Table 2 shows results with 15 pairs of sera from 12 previously vaccinated individuals (all N.B.L.) for whom data are available for all three tests. Previously, some individuals (e.g. Nos. 2, 5, 14) had received multiple boosters, others (e.g. Nos. 30, 32, 35) had received only the initial vaccination, usually about one year earlier. No response was seen in three persons (Nos. 2, 30, 37; 30 and 37 are also mentioned in Table 1), and a marginal response indicated by St-RIP and HA was seen in one individual (No. 35). Booster vaccinations had little or no effect on antibody levels in individual No. 5. The other persons showed varying responses in one or more of the tests.

DISCUSSION

To date, in the United States, the use of killed plague vaccine USP has been directed

TABLE 1. Antibody response of humans to initial plague vaccination as measured by three serologic tests. Pre-vaccination sera were obtained just before vaccination with 1 ml of vaccine and first post-vaccination sera approximately 1 month later; some individuals then received an additional 0.2 ml of vaccine and second post-vaccination sera were obtained 1-2 months later

Code	Serum	% St-RIP*		HA†	MPI‡
		1:10	1:100		
30	pre	14	11	<8	20
	post 1	16	11	<8	16
31	pre	15	11	<8	17
	post 1	72	44	32	6
32	pre	15	12	<8	20
	post 1	39	16	16	15
35	pre	9	—	<8	25
	post 1	63	28	16	10
37	pre	11	—	<8	17
	post 1	11	—	<8	19
	post 2	9	—	<8	14
44	pre	8	—	<8	34
	post 1	14	7	<8	26
	post 2	47	15	8	20
46	pre	9	—	<8	30
	post 1	28	10	32	22
	post 2	60	21	32	18
48	pre	11	—	<8	33
	post 1	10	7	16	33
	post 2	21	11	64	26

* % St-RIP = percentage of 125 I-labeled Fraction IB antigen bound by 10 μ l of serum at the indicated dilution in a staphylococcal radioimmune precipitin test

† HA = indirect hemagglutination, expressed as reciprocal of dilution

‡ MPI = mouse protection index

primarily toward the Armed Forces and personnel in high risk categories. It is noteworthy that military experience of vaccinated personnel in Vietnam strongly indicates the effectiveness of plague vaccine (Cavanaugh *et al.*, 1974). With reports of increasing incidents of plague in the Western United States the availability of vaccines and diagnostic tools may be increasingly important. As diagnostic tools, earlier studies suggested that the precipitin reaction (Larson, Philip, Wicht & Hughes, 1951) and the complement fixation reaction (Chen, Quan & Meyer, 1952) would be useful for

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TABLE 2. Antibody response of humans to booster plague vaccination as measured by three serologic tests. Pre-booster sera were obtained just before booster vaccination with 0.2 ml of vaccine and post-booster sera approximately 1-2 months later.

Code	Serum	% St-RIP*			HA†	MPI‡
		1:10	1:100	1:1 000		
2	pre	11	—	—	<8	18
	post	14	—	—	<8	20
5	pre	71	42	—	32	5
	post	73	52	21	32	8
5§	pre	73	53	22	32	11
	post	74	58	25	32	6
14	pre	74	40	14	256	19
	post	73	45	17	1024	10
24	pre	56	26	—	32	12
	post	65	66	33	4096	1
30	pre	8	—	—	<8	13
	post	14	—	—	<8	23
32	pre	24	9	—	8	22
	post	71	42	—	512	13
33	pre	47	18	—	64	14
	post	77	73	44	>8192	1
33¶	pre	77	59	20	4096	9
	post	76	59	20	>8192	13
35	pre	12	—	—	<8	26
	post	19	9	—	8	30
36	pre	44	18	6	128	23
	post	73	40	13	1024	6
37	pre	9	—	—	<8	19
	post	9	—	—	<8	17
37§	pre	11	—	—	<8	33
	post	10	—	—	<8	29
39	pre	7	5	—	8	24
	post	38	15	—	16	20
41	pre	60	29	9	32	7
	post	74	61	26	512	4

* % St-RIP = percentage of ¹²⁵I-labeled Fraction IB antigen bound by 10 µl of serum at the indicated dilution in a staphylococcal radioimmune precipitin test.

† HA = indirect hemagglutination, expressed as reciprocal of dilution.

‡ MPI = mouse protection index.

§ = 11 months after previous booster.

|| = Initial vaccine response in Table 1.

¶ = 20 months after previous booster.

detection of plague antigens in carcasses of animals suspected of harboring plague. The St-RIP reaction potentially offers a more rapid and sensitive procedure for the same purpose; we are able to quantitatively detect nanogram quantities of Fraction IB in tests where unlabeled antigen (purified or in vaccines) competes with labeled antigen (unpublished).

The finding that antibody assays by St-RIP correlate with other serologic tests demonstrates the reliability of St-RIP assay. St-RIP is very reproducible and highly sensitive, and the advantages over other serologic methods are several. MPI lacks quantitative precision, requires several days, and is impractical to run on numerous samples because of the amount of blood and numbers of animals needed. In contrast, St-RIP requires only a minute amount of blood, few reagents, and can be performed in a few hours. For reliability and reproducibility, HA must be run under very standardized conditions; in addition, some sera that show non-specific hemagglutination must be specially processed. Although a relationship between St-RIP and HA was seen in the majority of the sera, the finding of positive St-RIP and negative HA titers in 21 sera is puzzling. Six of these sera, from one individual, had HA titers ranging from 1:64 to 1:2048 in tests done in 1971. Prior data indicating positive HA titers were also available for four sera from two other individuals. We have no ready explanation for these discrepancies, but they may relate to storage conditions (e.g. repeated freezing and thawing). Storage conditions are a less likely cause for lack of correlation in other sera which were obtained more recently. Differences in reacting antibody populations might provide an explanation; monovalent antibodies, if present, might be bound to staphylococci in the St-RIP test, but not form bridges between red cells in the HA test.

It is evident from these studies that appreciable disagreement among the three tests may be seen with any individual serum. However, when groups are considered, there is very good agreement. As an indicator of plague antibody, St-RIP appears to be more reliable than the other tests, since the data would indicate fewer problems (e.g. attributable to interfering substances or storage conditions) with the St-RIP test. If limitations preclude multiple testing, St-RIP would appear to be the method of choice. Good correlations between HA titer and protection from fatal plague infections have been demonstrated for rats and monkeys and this may also apply to man (Williams & Cavanaugh, 1979). Since HA and St-RIP titer correlate well, the St-RIP titer probably also reflects protection against plague.

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) A staphylococcal radioimmune precipitation (St-RIP) test was applied to sera of laboratory personnel who had been vaccinated against plague. Antibodies were detected in the majority of 117 sera from 55 human vaccine recipients; a few individuals appeared to be immunologically nonresponsive since they failed to produce detectable antibodies. Highly significant statistical correlations were observed among St-RIP, indirect hemagglutination, and mouse protection index tests. Increases in antibody level were usually observed upon primary and booster vaccination.			

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